

Effect of binding on particle number fluctuations in a membrane channel

Alexander M. Berezhkovskii^{a)}

Mathematical and Statistical Computing Laboratory, CIT, National Institutes of Health, Bethesda, Maryland 20892

Mark A. Pustovoit

St. Petersburg Nuclear Physics Institute, Gatchina, 188350 Russia

Sergey M. Bezrukov^{b)}

Laboratory of Physical and Structural Biology, NICHD, National Institutes of Health, Bethesda, Maryland 20892 and St. Petersburg Nuclear Physics Institute, Gatchina, 188350 Russia

(Received 4 December 2001; accepted 17 January 2002)

Transport of solutes through membrane channels produces additional noise in the channel ion current because the number of solute molecules in the channel fluctuates. We obtain a general expression for the power spectral density of these fluctuations in a cylindrical channel in the presence of a binding site of arbitrary strength. The expression shows how the spectral density transforms from that in the case of no-binding to the Lorentzian spectral density corresponding to the strong-binding limit. Brownian dynamics simulations confirm our analytical results. © 2002 American Institute of Physics. [DOI: 10.1063/1.1458935]

I. INTRODUCTION

Membrane transport and molecular mechanisms of its regulation are among central themes of cell biology.¹ It is well established now that exchange of metabolites and other high-molecular-weight solutes between cells and subcellular compartments is facilitated by membrane-bound proteins that form channels of large (compared to ion channels of neurophysiology) diameter, up to several nanometers.² As a consequence, new methods for studies of channel-facilitated large-molecular transport are in great demand. Among several techniques there is a promising approach, the spectroscopy of metabolite-induced current fluctuations.^{3,4} It exploits the fact that the conductance of a pore filled with electrolyte decreases when a neutral particle enters into the pore. This principle has long been used in Coulter counters that count micron-sized particles in suspensions.⁵ Several years ago it was successfully applied to nanometer-sized polymer molecules passing through membrane channels.⁶ In a typical experiment, the measured quantity is the power spectral density (PSD) of fluctuations of the ionic current through a channel, $S_i(f)$. When the concentration of particles (metabolite molecules) is low, $S_i(f)$ is proportional to the normalized PSD of the particle number fluctuations $S(f)$: $S_i(f) = \langle N \rangle (\Delta g)^2 V^2 S(f)$, where $\langle N \rangle$ is the average number of particles in the channel, Δg is the reduction of the channel conductance caused by one particle, and V is the applied voltage. Experiments with different channels and particles show that at practically accessible frequencies of several kHz, the particle-induced noise exceeds the noise expected

from ion shot-noise⁷ by many orders of magnitude.⁴ Therefore, PSD is a source of information about the particle behavior in the channel.

In our previous paper⁸ we considered the particle number fluctuations in a cylindrical channel connecting two reservoirs and developed a theory that establishes the dependence of $S(f)$ on the particle diffusion constants in the channel and in the bulk as well as on the channel length and radius. That theory treats the problem in the case when particles diffuse freely in the channel. However, studies performed on channels of different origin,^{3,4,9–12} have demonstrated that usually there is a specific binding between channels and metabolites. When the binding is strong, that is, the mean lifetime of the particle on the site is much larger than the characteristic time of its diffusion through the channel, the particle transport is determined by binding and the channel conductance noise is of the generation-recombination type.^{13,14}

The goal of the present paper is to obtain a general expression for PSD of the particle number fluctuations in a membrane channel with a binding site of arbitrary strength. The theory includes both limiting cases described above, i.e., free diffusion and strong binding, and covers the gap between them. The main result of the paper is the expression for the spectral density $S(f)$ given in Eq. (16).

II. STATEMENT OF THE PROBLEM

Consider two compartments with noninteracting particles connected by a narrow cylindrical channel of length L (Fig. 1). The particles diffuse independently. In addition, they may be reversibly trapped by a binding site located in the middle of the channel. We assume that the concentration of the particles in the channel is sufficiently low so that competition of the particles for the site can be neglected. We also assume that the channel is so narrow that the particle reach-

^{a)}Permanent address: Karpov Institute of Physical Chemistry, Vorontsovo Pole 10, Moscow, K-64, 103064 Russia.

^{b)}Author to whom correspondence should be addressed: National Institutes of Health, Bldg. 9, Room 1E-122 Bethesda, MD 20892-0924. Electronic mail: bezrukov@helix.nih.gov; Tel: (301) 402-4701; FAX: (301) 402-9462.

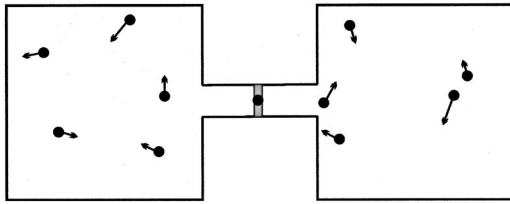


FIG. 1. A sketch of the system. Brownian particles wander freely (diffusion constant $D=0.5$) in two cubical reservoirs (side size 50) and cylindrical channel of length $L=40$ and diameter $a=5$. They bind occasionally on the site in the middle of the channel (shown as the gray strip) with the rate constants for trapping and releasing k_a and k_d . The total number of particles in the system is fixed, but the number of particles in the channel fluctuates. Arrows indicate the instantaneous particle displacements. There is no interaction between particles; any number of them can be on the site simultaneously.

ing the channel boundary from inside escapes forever (see Ref. 8 for more detailed treatment). Except for the binding site, the channel walls are assumed perfectly reflecting. We will calculate the time-dependent survival probability of a particle inside the channel given the equilibrium distribution of the particle initial position in the channel. Since this survival probability coincides with the normalized autocorrelation function of the number of particles in the channel, the former will be used to find the spectral density (see Ref. 8 for the details).

It is natural to approximate the particle motion in the channel as one-dimensional and characterize the channel by two parameters, the diffusion constant D and the channel length L . The binding site is described by two additional parameters: the association and dissociation rate constants, k_a and k_d . The probability density to find the diffusing particle at the point x , $0 < x < L$, at time t , $p(x, t)$, and its probability to be trapped at time t , $P_{tr}(t)$, satisfy

$$\begin{aligned} \frac{\partial p(x, t)}{\partial t} &= D \frac{\partial^2 p(x, t)}{\partial x^2} - k_a \delta\left(x - \frac{L}{2}\right) p(x, t) \\ &\quad + k_d \delta\left(x - \frac{L}{2}\right) P_{tr}(t), \\ \frac{dP_{tr}(t)}{dt} &= -k_d P_{tr}(t) + k_a p\left(\frac{L}{2}, t\right). \end{aligned} \quad (1)$$

To complete this set, we should add initial and boundary conditions:

$$\begin{aligned} p(0, t) &= p(L, t) = 0, \\ P_{tr}(0) &= \frac{k_a}{k_a + k_d L} = P_0, \\ p(x, 0) &= \frac{1}{L} [1 - P_{tr}(0)] = \frac{k_d}{k_a + k_d L} = p_0. \end{aligned} \quad (2)$$

The correlation function of interest (the survival probability), is given by

$$C(t) = P_{tr}(t) + \int_0^L p(x, t) dx. \quad (3)$$

The spectral density, being the cosine Fourier transform of $C(t)$, can be expressed in terms of the Laplace transform

$$\hat{C}(s) = \int_0^\infty e^{-st} C(t) dt,$$

where s is the Laplace parameter, as

$$S(f) = 4 \operatorname{Re}\{\hat{C}(2\pi fi)\}, \quad (4)$$

where i is the imaginary unit. Thus, the problem is reduced to finding the Laplace transform of the survival probability in the channel.

As we noted above, the spectral density is known in limiting cases of free diffusion (no binding site or very long channel) and strong binding (when the detrapping is the rate limiting step). In the first case we have⁸

$$S_\infty(f) = \frac{\sqrt{D}}{(\pi f)^{3/2} L} \frac{\sinh\left(\sqrt{\frac{\pi f}{D}} L\right) - \sin\left(\sqrt{\frac{\pi f}{D}} L\right)}{\sinh^2\left(\sqrt{\frac{\pi f}{D}} \frac{L}{2}\right) + \cosh^2\left(\sqrt{\frac{\pi f}{D}} \frac{L}{2}\right)}. \quad (5)$$

In the second limiting case the spectral density is^{13,14}

$$S_0(f) = \frac{4k_d}{k_d^2 + (2\pi f)^2}. \quad (6)$$

III. CALCULATIONS OF SPECTRAL DENSITY

The Laplace transform of $C(t)$ in Eq. (3) is

$$\hat{C}(s) = \hat{P}_{tr}(s) + \int_0^L \hat{p}(x, s) dx. \quad (7)$$

The Laplace transform $\hat{P}_{tr}(s)$ can be expressed in terms of $\hat{p}(x, s)$ using Eq. (1) together with the initial condition in Eq. (2)

$$\hat{P}_{tr}(s) = \frac{1 + (k_a + k_d L) \hat{p}\left(\frac{L}{2}, s\right)}{s + k_d} P_0. \quad (8)$$

It is convenient to express $\hat{p}(x, s)$ in terms of the Laplace transform of the propagator $g(x, t|x_0)$, that satisfies the diffusion equation

$$\frac{\partial g(x, t|x_0)}{\partial t} = D \frac{\partial^2 g(x, t|x_0)}{\partial x^2} \quad (9)$$

with the absorbing boundary conditions at the ends of the interval, $g(0, t|x_0) = g(L, t|x_0) = 0$, and the initial condition $g(x, 0|x_0) = \delta(x - x_0)$. With the aid of this propagator we can write the first equation of the set in Eq. (1) as

$$\begin{aligned} p(x, t) &= p_0 \int_0^L g(x, t|x_0) dx_0 + \int_0^t dt' g\left(x, t-t' \middle| \frac{L}{2}\right) \\ &\quad \times \left[k_d P_{tr}(t') - k_a p\left(\frac{L}{2}, t'\right) \right]. \end{aligned} \quad (10)$$

Laplace transform of Eq. (10) is

$$\hat{p}(x,s) = p_0 \int_0^L \hat{g}(x,s|x_0) dx_0 + \hat{g}\left(x,s\left|\frac{L}{2}\right.\right) \times \left[k_d \hat{P}_{tr}(s) - k_a \hat{p}\left(\frac{L}{2},s\right) \right]. \quad (11)$$

Using Eqs. (8) and (10), we can write $\hat{C}(s)$ in terms of $\hat{p}(L/2,s)$ and $\hat{g}(x,s|x_0)$:

$$\begin{aligned} \hat{C}(s) = & p_0 \int_0^L \int_0^L \hat{g}(x,s|x_0) dx dx_0 \\ & + \frac{P_0}{s+k_d} \left[1 + k_d \int_0^L \hat{g}\left(x,s\left|\frac{L}{2}\right.\right) dx \right] \\ & + \frac{k_a}{s+k_d} \left[1 - s \int_0^L \hat{g}\left(x,s\left|\frac{L}{2}\right.\right) dx \right] \hat{p}\left(\frac{L}{2},s\right). \end{aligned} \quad (12)$$

Next, we find an expression for $\hat{p}(L/2,s)$ in terms of $\hat{g}(x,s|x_0)$. For this purpose, we take $x=L/2$ in Eq. (11) and substitute there $\hat{P}_{tr}(s)$ given in Eq. (8). As a result, we obtain a linear equation with respect to $\hat{p}(L/2,s)$ from which we find

$$\begin{aligned} \hat{p}\left(\frac{L}{2},s\right) = & \frac{p_0}{s+k_d + s k_a \hat{g}\left(\frac{L}{2},s\left|\frac{L}{2}\right.\right)} \left\{ (s+k_d) \right. \\ & \left. \times \int_0^L \hat{g}\left(\frac{L}{2},s\left|x_0\right.\right) dx_0 + k_a \hat{g}\left(\frac{L}{2},s\left|\frac{L}{2}\right.\right) \right\}. \end{aligned} \quad (13)$$

Equations (12) and (13) provide an expression for survival probability in terms of $\hat{g}(x,s|x_0)$. The propagator can be found by solving Eq. (9):

$$\hat{g}(x,s|x_0) = \begin{cases} \frac{\sinh\left((L-x_0)\sqrt{\frac{s}{D}}\right) \sinh\left(x\sqrt{\frac{s}{D}}\right)}{\sqrt{sD} \sinh\left(L\sqrt{\frac{s}{D}}\right)}, & 0 < x < x_0; \\ \frac{\sinh\left((L-x)\sqrt{\frac{s}{D}}\right) \sinh\left(x_0\sqrt{\frac{s}{D}}\right)}{\sqrt{sD} \sinh\left(L\sqrt{\frac{s}{D}}\right)}, & x_0 < x < L. \end{cases} \quad (14)$$

The survival probability now reads:

$$\hat{C}(s) = \frac{1}{s} \left\{ 1 - \frac{2k_d}{k_a + k_d L} \cdot \left[\sqrt{\frac{D}{s}} \tanh\left(\frac{L}{2} \sqrt{\frac{s}{D}}\right) + \frac{2k_a}{4(s+k_d) \cosh^2\left(\frac{L}{2} \sqrt{\frac{s}{D}}\right) + k_a \sqrt{\frac{s}{D}} \sinh\left(L \sqrt{\frac{s}{D}}\right)} \right] \right\}. \quad (15)$$

Substituting the last expression into Eq. (4), we obtain the spectral density

$$\begin{aligned} S(f) = & \frac{4k_d}{\pi f(k_a + k_d L)} \cdot \text{Im} \left\{ \sqrt{\frac{D}{2\pi f i}} \tanh\left(\frac{L}{2} \sqrt{\frac{2\pi f i}{D}}\right) \right. \\ & \left. + \frac{2k_a}{4(2\pi f i + k_d) \cosh^2\left(\frac{L}{2} \sqrt{\frac{2\pi f i}{D}}\right) + k_a \sqrt{\frac{2\pi f i}{D}} \sinh\left(L \sqrt{\frac{2\pi f i}{D}}\right)} \right\}. \end{aligned} \quad (16)$$

This expression is the main result of the present paper. One can check that for limiting cases of very long and very short channels this general expression reduces to those in Eqs. (5) and (6).

Finally, we write an expression for PSD at zero frequency:

$$S(0) = \frac{k_d^2 L^3 + 3k_a k_d L^2 + 3k_a^2 L + 12k_a D}{3k_d D(k_a + k_d L)}. \quad (17)$$

In the limiting cases $S(0)$ is

$$S(0) = \begin{cases} \frac{L^2}{3D}, & k_d \rightarrow \infty, \text{ or } k_a \rightarrow 0, \text{ or } L \rightarrow \infty, \\ \frac{4}{k_d}, & L \rightarrow 0. \end{cases} \quad (18)$$

IV. SIMULATIONS

To confirm our analytical results we performed Brownian dynamics simulations of the system illustrated in Fig. 1. The number of particles in the entire system was fixed. The

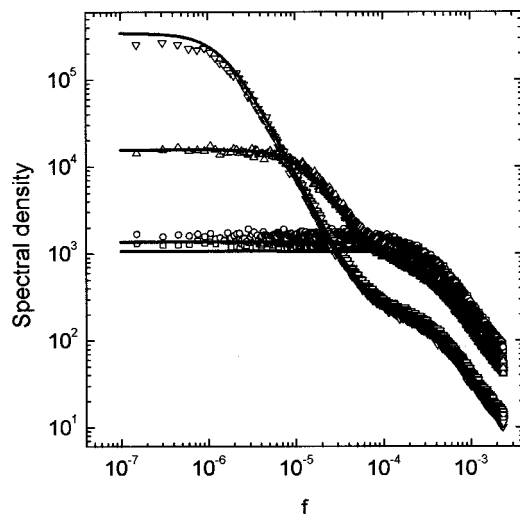


FIG. 2. The spectral density of particle number fluctuations obtained from simulations with fixed $k_a = 10^{-3}$ and values of k_d varying from bottom to top: ∞ (free diffusion), $9.91 \cdot 10^{-4}$, $9.97 \cdot 10^{-5}$, $1.01 \cdot 10^{-5}$. The solid curves display the analytical dependences in Eq. (16).

particles wandered randomly in three dimensions, interacting only with reflecting walls and the site. Interaction with the site was realized in the form of sticking and releasing probabilities ν_s and ν_r , so the actual values of k_a and k_d were obtained in the course of the simulations. The number of particles in the channel was recorded as time series, and the spectral density $S(f)$ was obtained by their Fourier transformation.

The results are shown in Fig. 2 (fixed k_a) and Fig. 3 (fixed k_d). The transition from diffusion-dominated behavior (the lower spectra) to a binding-dominated one (the upper ones) is clearly seen. The theoretical dependence given in Eq. (16) describes the spectra well in the full range of the parameters studied. There is a slight discrepancy in the diffusion-dominated case that is due to a finite channel diam-

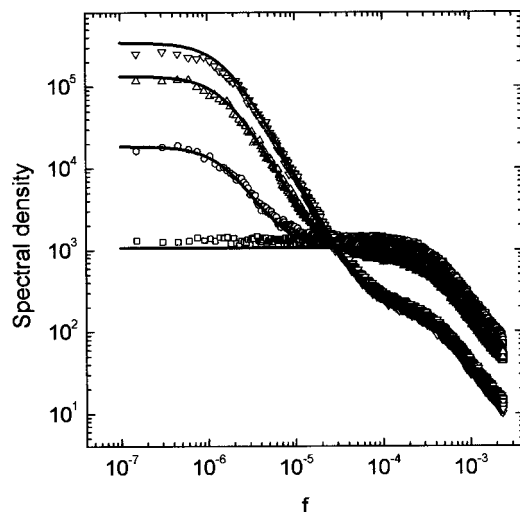


FIG. 3. Same as in Fig. 2 but with fixed $k_d = 10^{-5}$ and values of k_a varying from bottom to top: 0 (free diffusion, the same data as in Fig. 1), $9.35 \cdot 10^{-6}$, $9.96 \cdot 10^{-5}$, $1.01 \cdot 10^{-3}$. One can see that the characteristic “corner” frequencies of the Lorentzian part of the spectra are the same because of the same detrapping rate.

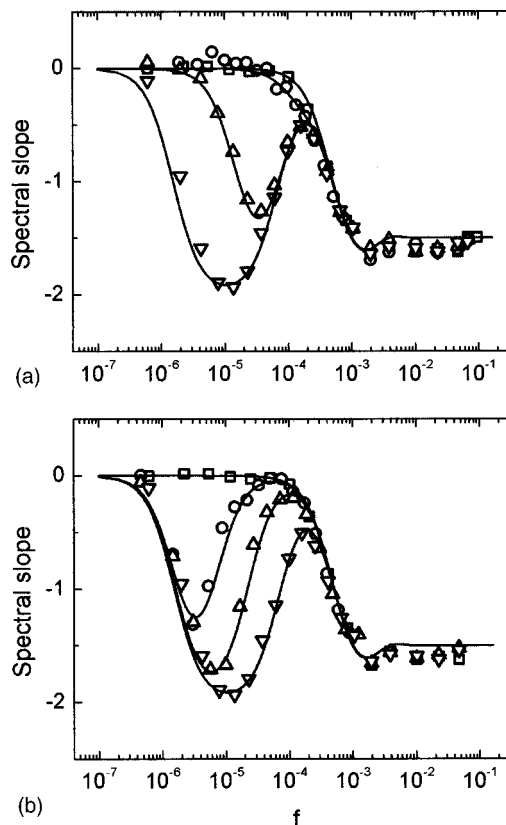


FIG. 4. The slope of the spectra shown in Fig. 2 [panel (a)], and Fig. 3 [panel (b)]. At high frequencies the slope is close to -1.5 . The Lorentzian part becomes pronounced in the case of strong binding. The solid curves are the slope found from the expression in Eq. (16). The symbols correspond to those used in Figs. 2 and 3.

eter (a detailed discussion of this issue can be found in Ref. 8). It is seen that $S(0)$ becomes close to its value in the strong binding limit in Eq. (18) already for $k_a \sim k_d L$.

The second informative quantity that can be easily obtained from the spectrum is its slope. It is defined as $d \ln S(f) / d \ln f$ and gives the apparent power-law exponent. We see that all the presented spectra demonstrate “universal diffusion behavior” $f^{-3/2}$ at high frequencies.¹⁵ When binding strengthens, the Lorentzian part appears and becomes more and more pronounced. Figure 4 displays this fact and shows also that the frequency range of the slope f^{-2} depends on the interplay between k_a and k_d .

V. CONCLUDING REMARKS

In the present paper we obtained an analytic expression for PSD of particle number fluctuations in a narrow membrane channel in the presence of a specific binding site, Eq. (16). Computer simulations demonstrated that the theory works well. Figures 2, 3, and 4 show how the presence of binding site influences the PSD low-frequency magnitude, shape, and slope. The expression for zero-frequency value of PSD given in Eq. (17) may be useful to analyze the data obtained in the case when the metabolite residence time in the channel is too small to be resolved by either real time or spectral shape analysis.

ACKNOWLEDGMENTS

One of the authors (M.A.P.) was also supported by the Russian State Programs on Physics of Quantum and Wave Processes, on Neutron Research of Matter and on Quantum Macrophysics.

¹*Cell Physiology*, edited by N. Sperelakis (Academic, New York, 1998).

²H. Nikaido, *Mol. Microbiol.* **6**, 435 (1992).

³S. Nekolla, C. Andersen, and R. Benz, *Biophys. J.* **66**, 1388 (1994).

⁴S. M. Bezrukov, *J. Membr. Biol.* **174**, 1 (2000).

⁵V. Kachel, in *Flow Cytometry and Sorting*, edited by M. R. Melamed, P. F. Mullaney, and M. L. Mendelsohn (Wiley, New York, 1979), pp. 61–104.

⁶S. M. Bezrukov, I. Vodyanoy, and V. A. Parsegian, *Nature (London)* **370**, 279 (1994).

⁷E. Barkai, R. S. Eisenberg, and Z. Schuss, *Phys. Rev. E* **54**, 1161 (1996).

⁸S. M. Bezrukov, A. M. Berezhkovskii, M. A. Pustovoit, and A. Szabo, *J. Chem. Phys.* **113**, 8206 (2000).

⁹T. Schirmer, T. A. Keller, Y.-F. Wang, and J. P. Rosenbusch, *Science* **267**, 512 (1995).

¹⁰C. Andersen, M. Jordy, and R. Benz, *J. Gen. Physiol.* **105**, 385 (1995).

¹¹L. Kullman, M. Winterhalter, and S. M. Bezrukov, *Biophys. J.* **82**, 803 (2002).

¹²T. K. Rostovtseva and S. M. Bezrukov, *Biophys. J.* **74**, 2365 (1998).

¹³K. M. Van Vliet and J. Blok, *Physica (Amsterdam)* **22**, 231 (1956).

¹⁴L. J. DeFelice, *Introduction to Membrane Noise* (Plenum, New York, 1981).

¹⁵M. Lax and P. Mengert, *Phys. Chem. Solids* **14**, 248 (1960).